How to Obtain Better Data From Zetasizer Measurements:

Solving Common Problems

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Contents

- Dynamic Light Scattering
  - Samples containing large particles/aggregates/contaminants
  - Data interpretation
- Zeta Potential
  - Concentration limits
  - High Conductivity
  - Data interpretation

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Sample Contains Large Particles/Aggregates/Contaminants

- Large particles/aggregates/contaminants can ruin a DLS measurement
- They may not be suitable for measurement by DLS and need to be removed from the sample before re-measurement
- Their presence can result in
  - Sedimentation
  - Number fluctuations

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Recognising Sedimentation

- Rate of sedimentation should be much slower than the rate of diffusion
- Sedimentation can be determined by checking the stability of the count rate from repeat measurements

File Edit View Measure Tools Security

Size

Records ... | Intensity ... | Volume P...

Record	Type	Angle °	Sample Name	Mean CR kcps
1	Size	173	Sample 1 Run 1	340.8
2	Size	173	Sample 1 Run 2	320.7
3	Size	173	Sample 1 Run 3	308.3

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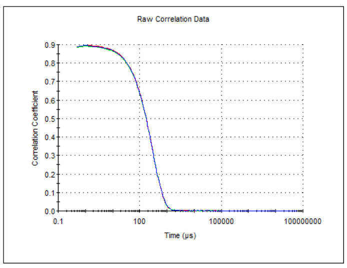
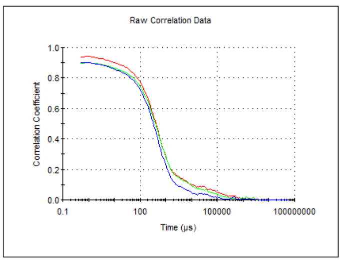
Recognising Sedimentation

- If the mean count rate decreases between repeat measurements, the sample will be changing each time it is measured
- Correlation functions from repeat measurements should perfectly overlap with each other
- If sedimentation is present (or the sample is changing with time), then the correlation functions will not overlap with each other





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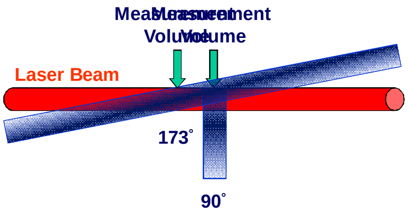
Number Fluctuations

- The presence of large particles/aggregates/ contaminants could cause number fluctuations in the DLS measurement
- Number fluctuations are defined as variations in the number of particles within the scattering volume during the course of a dynamic light scattering (DLS) measurement



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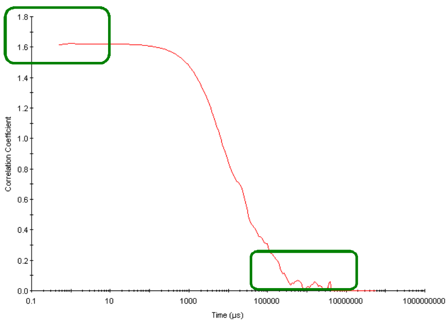
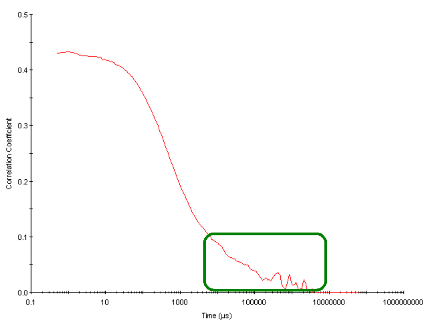
Recognising Number Fluctuations

- Number fluctuations are easy to identify and are typically manifested as elevated baselines in the correlation function
- In extreme cases, the measured intercepts are > 1





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Recognising Number Fluctuations:
Useful Parameters

- In Range
  - Overall indicator of data quality
  - Scaled from 0 to 100%
  - The higher the value the better
  - Values less than 90% will give a warning message in the Size Quality Report and Expert Advice System
- Measured Intercept
  - Signal to noise ratio obtained from the intercept of the Y-axis of the correlation function
  - Scaled 0 to 1
  - 1 = perfect correlation (impossible to achieve!)
  - Values > 1 caused by number fluctuations (poor definition of the baseline of the correlation function)

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How to Interpret DLS Data and Results

- The better the quality of the data, the more repeatable the answers obtained will be
- The quality of the data and results obtained can be determined by looking at various **Reports** and **Parameters** in the software

Recommended Reports

Report Name	Description
Intensity PSD	The primary result obtained from a DLS measurement
Size Quality	Incorporates a number of tests on any selected record
Correlogram	Shows the correlation coefficients determined at each delay time
Cumulants Fit	Shows the fit of the data points by the cumulants analysis from which the z-average diameter and polydispersity index are calculated according to ISO 13321
Distribution Fit	Shows the fit of the data points from the chosen distribution analysis from which the intensity size distribution is obtained
Expert System	Enables a quality check to be performed on 3 or more completed measurement records

Recommended Parameters

Parameter Name	Description
Mean Count Rate	The average count rate obtained during the course of the measurement
Attenuator	The attenuator position used for the measurement
Derived Count Rate	The count rate obtained taking into consideration the attenuation factor used
Cumulants Fit Error	The fit error obtained from the analysis of the data with cumulants
Multimodal Fit Error	The fit error obtained from the analysis of the data with either the general purpose or multiple narrow modes analysis
In Range	The overall quality of the data – a value less than 90% indicates the probable presence of number fluctuations due to the presence of large particles
Measurement Position	The position in the cuvette at which the measurement was taken

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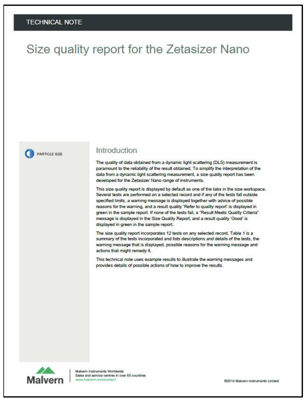
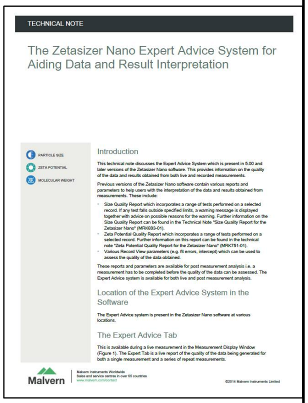
Further Information

- Technical Notes:
- Size quality report for the Zetasizer Nano
- The Zetasizer Nano Expert Advice System For Aiding Data and Result Interpretation





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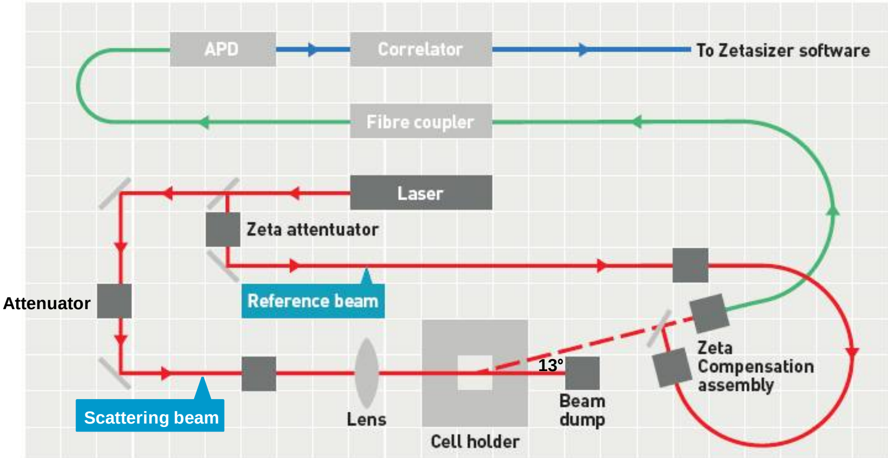
Laser Doppler Electrophoresis

- Scattered light detected at a forward angle of 13º
- Samples have to be low in turbidity



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Zeta Potential Cells

Cell Type				
Application				
Material				
Electrodes				
Minimum Volume				
Path Length				

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Zeta Potential Cells

Cell Type	Folded Capillary Cell 	Dip Cell 	High Conc^n Cell 	Surface Zeta Potential Cell 
Application	Aqueous	Aqueous/Non-Aqueous	Aqueous	Flat Surface Aqueous
Material	Polycarbonate	Plastic or Glass Cuvette	Glass	Plastic or Glass Cuvette
Electrodes	Gold Coated	Palladium	Palladium	Palladium
Minimum Volume	50µl (DBM)	750µl	100µl	750µl
Path Length	4mm	10mm	1.5mm	10mm

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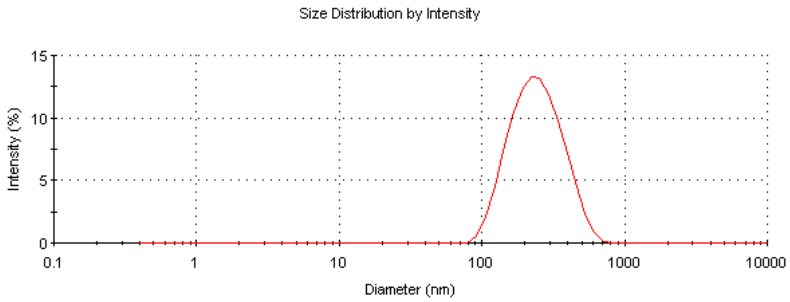
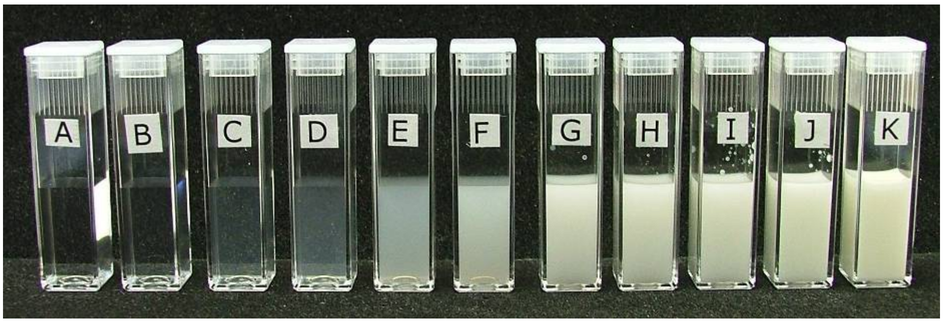
Case Study: Intralipid

- This is a commercial oil in water emulsion sample with a mean size of around 220nm
- The intensity size distribution from a dynamic light scattering measurement obtained at 0.01% w/v concentration is shown



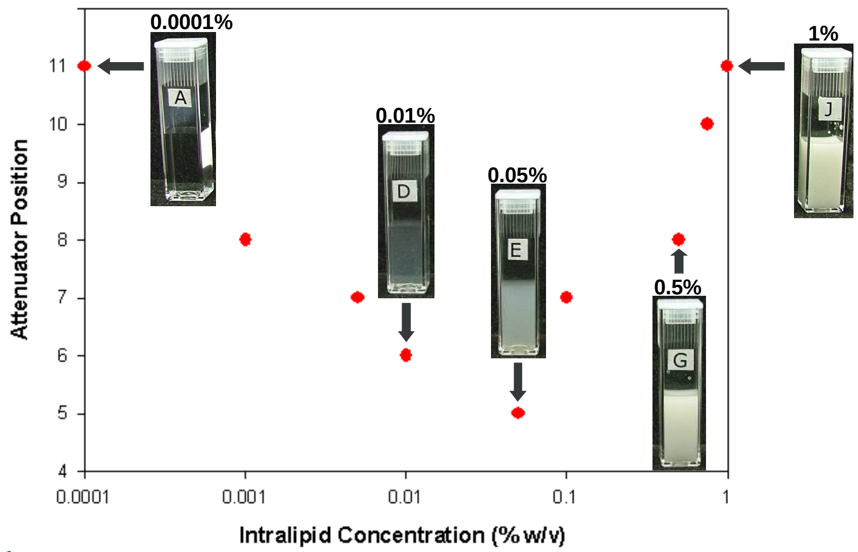
Case Study: Intralipid



A = 0.0001% w/v
B = 0.001% w/v
C = 0.005% w/v
D = 0.01% w/v
E = 0.05% w/v
F = 0.1% w/v

G = 0.5% w/v
H = 0.75% w/v
I = 1% w/v
J = 2% w/v
K = 10% w/v (neat sample)

Case Study: Intralipid



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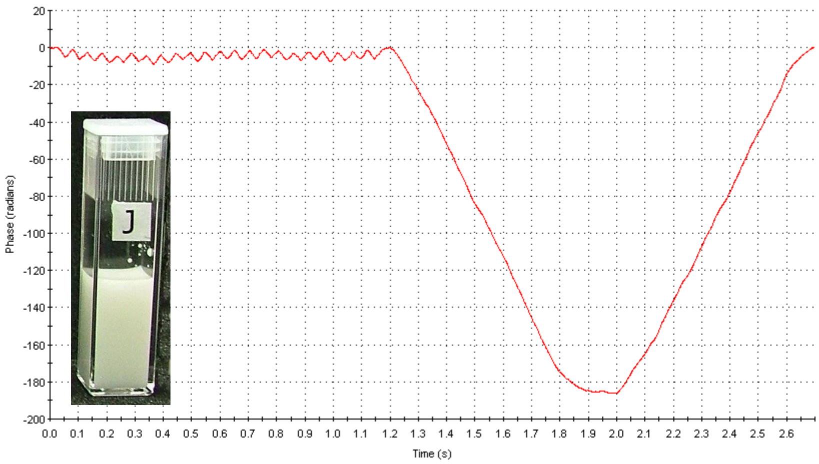
Case Study: Intralipid Phase Plots

Intralipid 1% w/v sample



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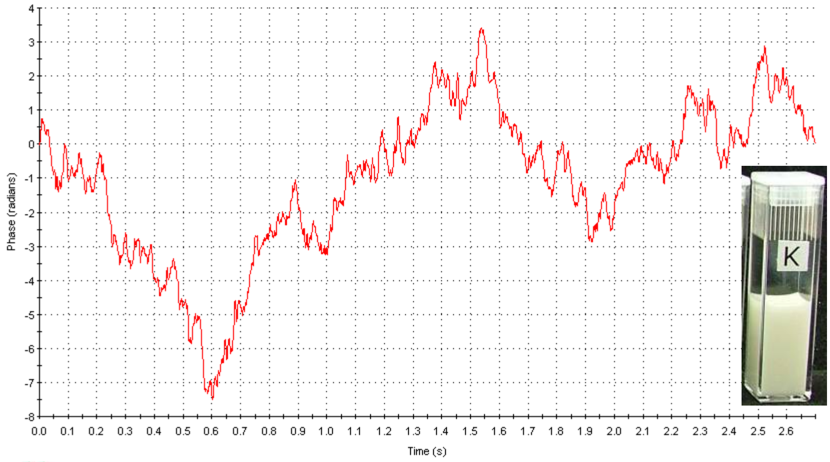
Case Study: Intralipid Phase Plots

Intralipid 2% w/v sample



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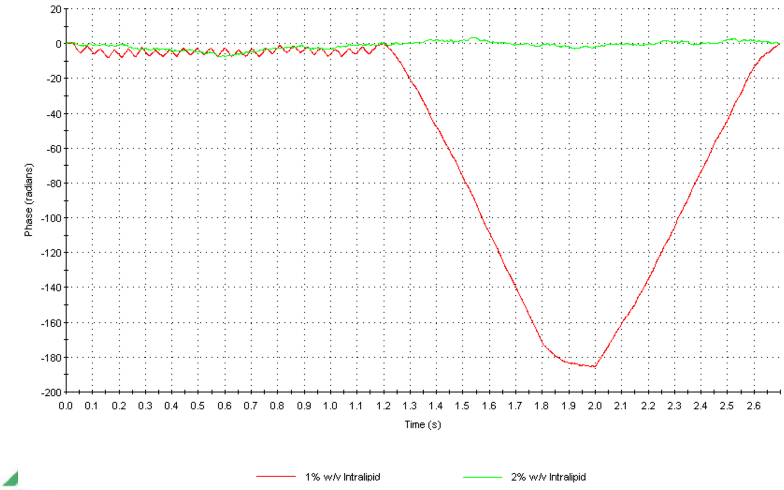
Case Study: Intralipid Phase Plots

Intralipid 1 and 2% w/v samples overplotted



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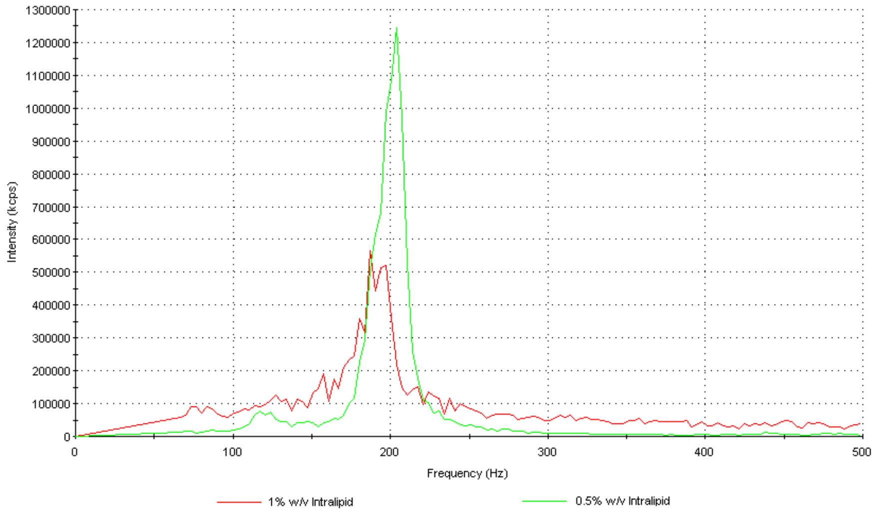
Case Study: Intralipid Frequency Plots



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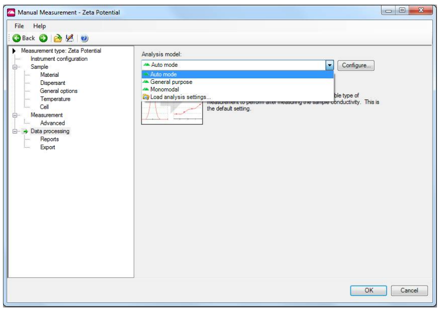
Use Auto Mode

- Default analysis is Auto Mode which selects the most appropriate model and conditions for the measurement from the measured conductivity
- The automatic settings of the instrument try to minimize these potential problems as much as possible

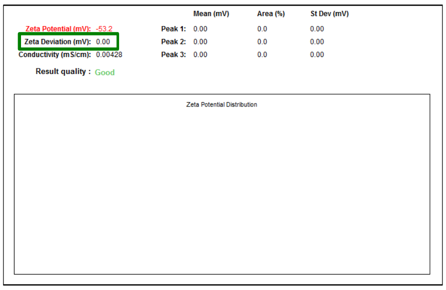


Ensure Monomodal Has Been Used

- Two models are available
  - General Purpose
  - Monomodal
- General Purpose uses a longer application of a steady field giving a higher degree of electrode polarization (reducing cell and sample life)
- Monomodal used for conductivities greater than 5mS/cm
- It is worth ensuring that Monomodal was used as this will minimize sample/electrode degradation and electrode polarization effects

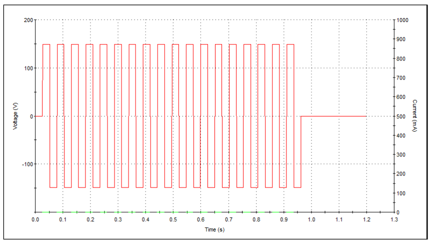
Ensure Monomodal Has Been Used

Zeta Potential Report



No zeta potential distribution
Zeta Deviation = 0

Voltage/Current Report



FFR Only

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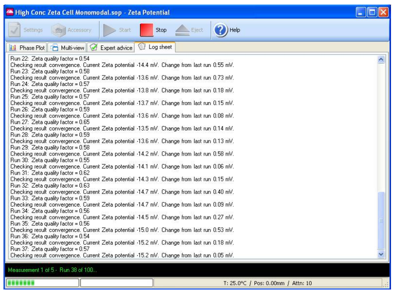
Follow the Quality Factor During the Measurement

- The quality factor is a signal to noise based parameter which determines the quality of the phase data obtained from the measurement
- After a minimum number of sub runs (10 by default), the software checks the quality factor and this can be followed in the Log Sheet tab of the Live Measurement Window
- The result is reliable when the result quality reaches 1



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Follow the Quality Factor During the Measurement

- For some high conductivity samples, the quality factor may initially show a gradual increase over the first 30 or so sub runs, but may never reach a value of 1
  - Particularly for samples whose zeta potential is low or at zero
- Continued sub run accumulation can then result in sample/electrode degradation causing the quality factor to decrease
  - Recommended to manually define the duration (i.e. number of sub runs) to stop the measurement before this point is reached
  - Experimentally determine the optimum number of sub runs to be used

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Limit the Number of Sub Runs to be Used

- For some samples, it is recommended to manually specify the number of sub runs per measurement
- Limiting the number of sub runs per measurement should help to minimize electrode polarization/degradation and sample degradation
  - It should be noted that this approach may result in data where the quality factor does not reach a value of 1
- There is a trade off between the quality of the data obtained and minimizing sample degradation
- The most appropriate number of sub runs defined per measurement should be determined from a series of experiments on the sample

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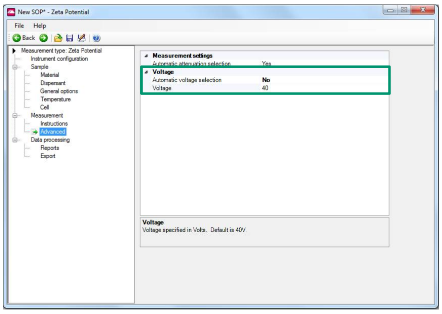
Manually Reduce the Voltage

- In Auto Mode, the voltage is automatically selected for the zeta potential measurement based on the measured conductivity
- The applied voltage is automatically reduced as the conductivity increases to limit the current and hence extend the life of the cell and sample
- However it may be necessary to further manually reduce the voltage
- Setting the selection to "No" will allow manual selection of the applied voltage

Conductivity (mS/cm)	Voltage Selected (V)
<5	150
5 to 30	50
>30	10



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Limit the Number of Repeat Measurements on a Sample

- For some high conductivity samples, it may be necessary to reduce the number of repeat measurements performed on a sample
- It is recommended to use a maximum number of 3 repeat measurements to try and minimize sample/electrode degradation and extend the life of the folded capillary cell
- There will be a trade off between the repeatability of the results obtained and minimizing sample degradation

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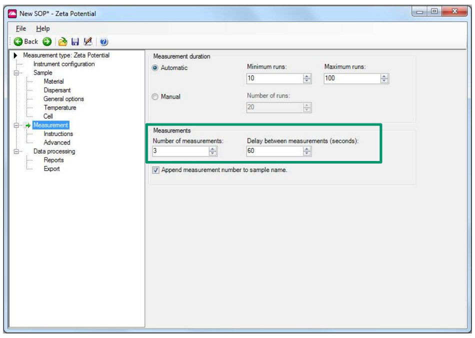
Include a Pause Between Repeat Measurements

- Improved repeatability can be achieved by including a pause between repeat measurements
- Typical pause durations of 30 or 60 seconds are recommended
- This can be set in the Measurement Setup



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Use the Diffusion Barrier Method (DBM)

- In the diffusion barrier technique, a small plug of sample (e.g. 20 to 100µL) is introduced into a folded capillary cell containing the same buffer that the sample is prepared in, and is therefore isolated from the electrodes
- The physical distance between the sample and the electrodes means that the sample is protected
- The DBM has two advantages
  - The sample is never in contact with the electrodes
  - A much smaller volume of sample is required
- The DBM should be used for any high conductivity sample (protein or colloid)

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Use the Diffusion Barrier Method (DBM)

Normal fill of a folded capillary cell

Fill cell with sample dispersant only

Gel electro-phoresis loading tips

Introduce a small aliquot of sample

Sample ready for measurement

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- The better the quality of the data, the more repeatable the answers obtained will be
- The quality of the data and results obtained can be determined by looking at various **Reports** and **Parameters** in the software

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Recommended Reports

Report Name	Description
Zeta Potential	The zeta potential result obtained from the measurement
Zeta Quality	Incorporates a number of tests on a selected record
Expert Advice	Quality checks on a single record and trends 3 or more records
Phase	The phase difference between the measured beat frequency and the reference frequency plotted as a function of time
Frequency	The frequency spectrum obtained from the SFR part of the measurement
Voltage and Current	The voltage applied and the current detected in the cell over the duration of the measurement

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Recommended Parameters

Parameter Name	Description
Zeta Potential Mean	The mean zeta potential value
Zeta Potential Width	The standard deviation of the zeta potential distribution
Conductivity	The conductivity of the sample determined from the measurement
Attenuator	The attenuator position used during the measurement
Quality Factor	A signal to noise based parameter derived from a phase analysis during the FFR stage of the measurement
SFR Spectral Quality	A signal to noise based parameter that is derived from the frequency analysis during the SFR stage of the measurement
Effective Voltage	The voltage requested during the measurement
Zeta Runs	The number of sub runs used in the measurement

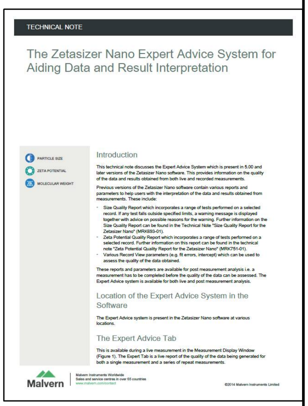
Further Information

- Technical Notes:
- Zeta potential quality report for the Zetasizer Nano
- The Zetasizer Nano Expert Advice System For Aiding Data and Result Interpretation

TECHNICAL NOTE

Zeta potential quality report for the Zetasizer Nano

Introduction



TECHNICAL NOTE

The Zetasizer Nano Expert Advice System for Aiding Data and Result Interpretation

Introduction

Many thanks for your attention

Any questions?

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